This protocol describes a method for titering and determining coreceptor usage of viral isolates. The method uses three different cell types that either express no added coreceptor (U373-MAGI) or express CXCR4 alone (U373-MAGI-CXCR4cem) or CCR5 alone (U373-MAGI-CCR5e) in the presence of CD4 and the indicator gene. Infected cells and syncytia are easily visualized under light microscopy by their blue nuclear staining.

Reagents

Cells	U373-MAGI and derivatives U373-MAGI-CXCR4 and U373-MAGI-CCR5e (Catalog #3595, 3596, and 3597, respectively).
Test Virus	HIV-1, HIV-2, or SIVmac strains that use either CXCR4 or CCR5 as coreceptors.
DEAE Dextran	(Pharmacia, Catalog #17-0350-01), prepared at 200 μg/ml in DMEM
Culture Medium	DMEM 90%; fetal bovine serum, 10%.
Fixing Solution	1% formaldehyde, 0.2% glutaraldehyde (in PBS). This solution can be made up in advance and stored at 4°C in the dark for approximately 1 month.
Staining Solution	To prepare 1.0 ml of Staining Solution, combine 950 μl PBS, 20 μl 0.2 M potassium ferrocyanide, 20 μl 0.2 M potassium ferricyanide, 1.0 μl 2.0 M Mg_2Cl, and 10 μl X-gal Stock.
X-Gal Stock	Prepare at 40 mg/ml in DMSO. X-gal stock should be stored in the dark at -20°C. It will turn yellow over time, but this does not affect the assay. Discard the stock if it becomes greenish-brown.

Procedure

1. Plate U373-MAGI cells at 0.6 x 10^5 cells per well (24 well plate) or at 1.2 x 10^5 cells per well (12 well plate). The cells should be 30% confluent one day after plating.

2. One day after plating out the cells, prepare dilutions of test virus in culture medium. Remove the culture medium from the plated cells and add 150 μl of virus to each 6 mm well (300 μl if a 12 well plate is used). Virus samples should be tested in duplicate. Add DEAE Dextran to each well at a final concentration of 20 μg/ml.

3. Allow the virus to adsorb for at least 2 hours in a 37°C, 5% CO_2 incubator. Rock the plates every 45 minutes to prevent the cells from drying.

4. After the adsorption period, add 1-2 ml of fresh culture medium to each well. It is not necessary to remove the viral inoculum. Incubate the cells 40-48 hours in a 37°C 5% CO_2 incubator. The cells should be just subconfluent.

5. Remove the culture medium and add 1-2 ml of fixing solution to each well. Incubate for exactly 5 minutes at room temperature. β-galactosidase activity decreases dramatically if the fixing solution is left on for more than 10 minutes.

6. Remove the fixing solution and wash the cells twice with PBS. Add enough staining solution to each well to just cover the cells. Incubate the cells at 37°C for two hours. Do not extend the incubation period or background staining will occur. NOTE THAT THE INCUBATION TIME FOR THE U373-MAGI CELLS IS LONGER THAN THAT FOR THE HELA-MAGI CELLS.

7. Wash the plates twice with PBS. Positive syncytia will stain blue. Most staining will be primarily in the nuclear region because the ß-gal gene has been modified with the SV40 nuclear localizing

sequence. Count the number of blue-stained cells. Titration values are expressed as the number of stained cells multiplied by the viral dilution.

8. Plates can be stored in PBS with sodium azide if a permanent record is desired. The color will not fade if the plates are kept from strong sunlight.

Alternate Method for Titering Infected Cells:

Dilutions of infected cells, rather than cell-free virus, can be plated onto the U373-MAGI cells if desired. Prepare serial dilutions of infected test cells in culture medium starting with 1×10^5 cells. If infected cells are used, do not add DEAE Dextran to the cultures. It is not necessary to wash off unattached cells from the monolayer.